Constrictive Effect of Thyroxine on the Ductus Arteriosus in Fetal Rats

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ABSTRACT. This work was conducted to know whether thyroxine (T_4) when injected into fetal rats would induce a constriction of the ductus arteriosus (DA). In Experiment 1, fetal rats on day 20 of gestation were given a subcutaneous injection of 1 or 10 μ g T_4 through the maternal uterine wall, and were autopsied 3 or 6 hr later. Similarly, in Experiment 2, the mother rats were given an injection of hydrocortisone (10 mg/kg) just after fetal T_4 injections. In either series of experiments, uninjected littermates served as controls. According to the whole-body freezing and shaving method, the DA was exposed and calibrated under a dissecting microscope. In Experiment 1, the DAs of the T_4 -injected fetuses, 3 hr later, were significantly smaller in caliber than those of their controls, but recovered 6 hr later. In Experiment 2 with fetal T_4 injection followed by maternal hydrocortisone injection, the DAs of the T_4 -injected fetuses were further smaller in caliber than those of their controls which were clearly shrunken as compared with those of controls in Experiment 1. These results indicate that T_4 exerts a constrictive effect on the DA, an effect which is strengthened in the presence of hydrocortisone.—**KEY WORDS:** ductus arteriosus, fetus (rat), thyroxine.

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The ductus arteriosus (DA) is patent during the prenatal period and conducts most of the blood flowing from the pulmonary artery to the aorta, thus forming a part of the typical fetal circulation. The DA is increased in caliber toward the end of gestation, but after birth with the beginning of respiration, it is rapidly closed to remain as a fibrous cord. The DA has very specific properties. For example, the patency of this vessel is maintained by prostaglandins [3, 5, 14], but it is disturbed by glucocorticoids [15, 21], this latter effect being due to the inhibition of prostaglandin biosynthesis [12, 13].

Glucocorticoids and thyroid hormones play a regulatory role in the adaptation of the fetal respiratory system to the forthcoming extrauterine life after birth. Deficiency of glucocorticoids causes maldevelopment of the fetal lung and immaturity of the pulmonary surfactant system, thus constituting one of the major etiological factors leading to the respiratory distress syndrome (RDS) [6]. Treatment with glucocorticoids accelerates the maturation of the surfactant system [9]. Thyroxine (T_4), a thyroid hormone, also promotes the maturation of this system; in particular, this promotion by T_4 is further enhanced in the presence of glucocorticoids [11]. In addition, it has been reported that triiodothyronine receptors exist in the rat lung [16], and that T_4 causes an increase in the level of pulmonary glucocorticoid receptors [17].

Considering the foregoing facts together with a report that the incidence of the patent DA is extremely high in the human infants with RDS [20], it can be speculated that thyroid hormones also exert a constrictive effect on the DA. Accordingly, the present work was designed to test this speculation by the use of T_4 as a thyroid hormone. In the present work, T_4 was injected directly into fetal rats *in utero*, since it has been known that T_4 given to the mother fails to reach the fetus through the placenta [10, 19].

MATERIALS AND METHODS

Female Wistar rats, 12–15 weeks old at the time of mating, were used in this work. They were given a commercial diet (Labo MR Breeder) and tap water, both *ad libitum*, and were kept at a room temperature of $22\pm3^\circ\text{C}$ with a relative humidity of $55\pm10\%$ in a 12:12 hr photoperiod. The females were placed with males overnight and examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was found was designated as day 0 of gestation, and the females were caged individually from this time.

The following two series of experiments were carried out on day 20 of gestation.

In the first series of experiments, the pregnant rats were subjected to mid-ventral laparotomy under ether anesthesia. Then 2 or 3 fetuses in a uterine horn in each litter were given a subcutaneous injection of 1 or 10 μ g T_4 (Sigma) dissolved in 0.05 ml saline, through the uterine wall. The dose of T_4 was according to that used for neonatal rats [7, 9]. Autopsy was performed 3 or 6 hr later.

In the second series of experiments, the pregnant rats were similarly operated. Just after an injection of 10 μ g T_4 into 2 or 3 fetuses each in a uterine horn, the mother was given a subcutaneous injection of 10 mg/kg hydrocortisone (Sigma), the dose being according to Momma *et al.* [15]. Autopsy was performed 3 hr later.

At autopsy, in either series of experiments, 2 or 3 uninjected littermates in the contralateral uterine horn in each litter served as controls. Each of the fetuses obtained was rapidly frozen in an acetone-dry ice mixture, and then was stored for several days at -20°C prior to observation. The caliber of the DA was determined by the whole-body freezing and shaving method described elsewhere [2].

Statistical analyses of data were made with Student's

t-test. A *P* value less than 0.05 was considered to be statistically significant.

RESULTS

In the first series of experiments, the DAs of the T_4 -injected fetuses, whether the dose was 1 μ g or 10 μ g, were significantly smaller in caliber than those of their respective controls 3 hr later, but recovered 6 hr later (Fig. 1). Morphologically, the DA was shrunken with its wall

Fig. 1. Changes in caliber of the ductus arteriosus of fetal rats 3 or 6 hr after thyroxine (T_4) injection. (A), Experiment 1 in which pregnant mother rats were laparotomized without maternal injection. (B), Experiment 2 in which laparotomized pregnant rats were given 10 mg/kg hydrocortisone injection. Open columns show uninjected controls, hatched columns 1 μ g T_4 -injected fetuses, and shaded columns 10 μ g T_4 -injected fetuses. Each column represents mean $\pm$ SEM of 12 fetuses from 4–5 litters. *, significantly different from the control in the same group ($P < 0.05$). There are significant differences between the two # marks and between the two + marks ($P < 0.05$).

which was thicker and more whitish in appearance than that in the control (cf. A and B in Fig. 2).

In the second series of experiments, with fetal T_4 injection followed by maternal hydrocortisone injection, the DAs of the T_4 -injected fetuses were further smaller in caliber than those of their controls which were clearly shrunken as compared with those of corresponding controls in the first series of experiments (Fig. 1).

DISCUSSION

The foregoing observations first revealed that T_4 when injected directly into a fetal rat could induce a significant constriction of the DA 3 hr later, suggesting that thyroid hormones are associated with the constriction of the DA. Nevertheless, the DA recovered 6 hr later, indicating that the constriction thus induced is merely a temporary change unless the effect of the hormone is prolonged. In our previous study [2], similar results were also obtained by indomethacin, a synthetic compound having a nature similar to that of glucocorticoids. In the second series of experiments in the present study, maternally-given hydrocortisone induced a significant constriction of the fetal DA 3 hr later, well in harmony with the finding of Momma *et al.* [15].

The foregoing observations further revealed that, in the presence of hydrocortisone, T_4 injected into fetuses could induce a constriction of the DA to a greater degree than in the absence of hydrocortisone. Accordingly, it can be surmised that the combined administration of thyroid hormone and glucocorticoid enhances the constrictive effect on the fetal DA.

As stated in the introduction, the constrictive effect of glucocorticoids on the DA is said to be due to the inhibition of prostaglandin biosynthesis. If the patency of the DA is maintained only by prostaglandins, it would

Fig. 2. Sections showing the DAs of a control fetus (A) and a thyroxine (T_4)-injected fetus (B) in the same litter. T_4 was injected at a dose of 10 μ g. Arrows indicate the DAs. The DA in B is smaller in caliber than that in A. Ao, aorta; LL, left lung; T, thymus; RL, right lung. $\times 10$.

likely follow that thyroid hormones also have a kind of inhibitory actions on prostaglandin biosynthesis, though there has been so far neither substantial nor circumstantial evidence regarding this matter.

Incidentally, both glucocorticoids and thyroid hormones promote the maturation of the pulmonary surfactant system in favor of switching the gas-exchange from the placental to the pulmonary route. In this sense, it would be pertinent to consider that the patency of the DA is regulated by a balance between the opposing actions of prostaglandin E₂ and oxygen [4]. Furthermore, it has been shown that the ligation of maternal uterine blood vessels or of umbilical cords in the rat causes a rise in both the concentration of fetal plasma corticosterone [8] and the implicated amount of fetal pulmonary surfactant [1], the ligation implying the decline of oxygen content in blood. In addition, thyroid hormones are known to be a factor to increase the rate of oxygen consumption [18]. Although these findings seem to imply that the constrictive action of thyroid hormones and glucocorticoids on the DA takes place through changes in the oxygen consumption as well, the response of the DA to these hormones may also occur through a variety of other factors to be surveyed in future.

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